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Differential effect of T-type voltage-gated Ca^{2+} channel disruption on renal plasma flow and glomerular filtration rate in vivo

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[1]*Cardiovascular and Renal Research, University of Southern Denmark, Odense, Denmark;* [2]*Department of Urology, Odense University Hospital, Odense, Denmark; and* [3]*Clinical Pathology, Odense University Hospital, Odense, Denmark*

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Thuesen AD, Andersen H, Cardel M, Toft A, Walter S, Marcussen N, Jensen BL, Bie P, Hansen PB. Differential effect of T-type voltage-gated Ca^{2+} channel disruption on renal plasma flow and glomerular filtration rate in vivo. *Am J Physiol Renal Physiol* 307: F445–F452, 2014. First published June 25, 2014; doi:10.1152/ajprenal.00016.2014.—Voltage-gated Ca^{2+} (Ca_v) channels play an essential role in the regulation of renal blood flow and glomerular filtration rate (GFR). Because T-type Ca_v channels are differentially expressed in pre- and postglomerular vessels, it was hypothesized that they impact renal blood flow and GFR differentially. The question was addressed with the use of two T-type Ca_v knockout ($Ca_v3.1^{-/-}$ and $Ca_v3.2^{-/-}$) mouse strains. Continuous recordings of blood pressure and heart rate, para-aminohippurate clearance (renal plasma flow), and inulin clearance (GFR) were performed in conscious, chronically catheterized, wild-type (WT) and $Ca_v3.1^{-/-}$ and $Ca_v3.2^{-/-}$ mice. The contractility of afferent and efferent arterioles was determined in isolated perfused blood vessels. Efferent arterioles from $Ca_v3.2^{-/-}$ mice constricted significantly more in response to a depolarization compared with WT mice. GFR was increased in $Ca_v3.2^{-/-}$ mice with no significant changes in renal plasma flow, heart rate, and blood pressure. $Ca_v3.1^{-/-}$ mice had a higher renal plasma flow compared with WT mice, whereas GFR was indistinguishable from WT mice. No difference in the concentration response to K^+ was observed in isolated afferent and efferent arterioles from $Ca_v3.1^{-/-}$ mice compared with WT mice. Heart rate was significantly lower in $Ca_v3.1^{-/-}$ mice compared with WT mice with no difference in blood pressure. T-type antagonists significantly inhibited the constriction of human intrarenal arteries in response to a small depolarization. In conclusion, $Ca_v3.2$ channels support dilatation of efferent arterioles and affect GFR, whereas $Ca_v3.1$ channels in vivo contribute to renal vascular resistance. It is suggested that endothelial and nerve localization of $Ca_v3.2$ and $Ca_v3.1$, respectively, may account for the observed effects.

afferent arteriole; efferent arteriole; kidney arteries

THE REGULATION of renal blood flow, including renal medullary blood flow, and glomerular filtration pressure involves voltage-gated Ca^{2+} (Ca_v) channels, which mediate changes in pre- and postglomerular vessel diameters. These effects influence glomerular filtration rate (GFR) and salt and water homeostasis and thereby blood pressure.

Ca_v channels are differentially expressed in the renal vasculature, as preglomerular vessels express L-type ($Ca_v1.2$) and T-type ($Ca_v3.1$ and $Ca_v3.2$) Ca^{2+} channels, whereas postglomerular cortical efferent vessels are devoid of L-type Ca^{2+} channels, but mouse efferent arterioles express T-type ($Ca_v3.1$ and $Ca_v3.2$) Ca^{2+} channel subunits (17, 36). In contrast, both L-type and T-type Ca^{2+} channels are found in thick muscular juxtamedullary efferent arterioles, which control medullary perfusion (17).

The involvement of T-type Ca^{2+} channels in the regulation of microvascular diameter has been observed in both rat and mouse afferent and efferent arterioles (12, 17, 35, 36), whereas L-type Ca^{2+} channels are assumed not to play a role in cortical efferent arteriolar contractility (6, 17, 28, 31). Wheras mouse studies have indicated a depolarization-induced effect in efferent arterioles, results from rats are less clear. Some studies have shown that Ca^{2+} influx pathways in rat efferent vessels are not dependent on depolarization for the ANG II-induced contraction (24, 28, 29), whereas others have shown that the T-type blockers mibefradil and nickel chloride affect the ANG II-elicited constriction in efferent arterioles of the isolated perfused hydronephrotic rat kidney model (21, 35). Moreover, there appears to be regional differences in that juxtamedullary arterioles (and vasa recta) exhibit a larger dependence on depolarization compared with cortical efferent arterioles (17). In vivo data from dogs treated with channel antagonists have confirmed the heterogeneity between afferent and efferent arterioles as well as an important contribution from T-type Ca^{2+} channels in efferent arterioles (23, 42). L-type antagonists led to an increased filtration fraction, corresponding to dilatation of afferent but not efferent arterioles, whereas T-type antagonists caused no increase in the filtration fraction (23) but a similar increase in renal blood flow as L-type antagonists. The contribution of T-type Ca^{2+} channel activity to efferent constriction is likely supported by the $Ca_v3.1$ subtype (36). The $Ca_v3.2$ subtype is involved in nitric oxide (NO)-dependent dilatation of efferent arterioles since $Ca_v3.2$ and endothelial NO synthase (eNOS) blockade inhibited the secondary dilatation after a depolarization-induced constriction of perfused efferent arterioles (36). This is corroborated by the finding that $Ca_v3.2$-deficient mice exhibit normal vasoconstrictor responses in coronary arteries but reduced relaxation of coronary arterioles after the administration of ACh and nitroprusside (8). Based on these in vitro observations and the intrarenal vascular distribution of Ca_v3 subtypes, it was hypothesized that disruption of $Ca_v3.1$ would increase renal blood flow but not alter GFR proportionally since it is expressed both in pre- and postglomerular vessels. On the other hand, targeting $Ca_v3.2$ would be predicted to increase vascular resistance and filtration fraction due to its preferential efferent localization and NO-promoting action in vitro.

Use of mice with targeted disruption of Ca_v channels allows the investigation of the importance of T-type Ca^{2+} channels

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without the question of drug specificity. To examine the working hypotheses, we investigated renal function in conscious, unstressed freely moving mice that were deficient in T-type $Ca_v3.1$ and $Ca_v3.2$ channels ($Ca_v3.1^{-/-}$ and $Ca_v3.2^{-/-}$ mice) by infusion of inulin and para-aminohippurate (PAH) through chronic indwelling catheters.

MATERIALS AND METHODS

Animals. Experiments were conducted in $Ca_v3.2^{-/-}$ mice and their wild-type (WT) littermates ($Ca_v3.2^{+/+}$ mice) obtained from heterozygous breeding (Mutant Mouse Regional Resource Centers, Columbia, MO). Mice were on a mixed 129 and C57BL/6J background. Furthermore, $Ca_v3.1^{-/-}$ mice (27) and C57BL/6J WT mice (Taconic Farm, Ry, Denmark) were used. C57BL/6J WT mice were used as controls as $Ca_v3.1^{-/-}$ mice were backcrossed to a C57BL/6J background for >10 generations. Animals were 8–10 wk of age and of both sexes. The experimental protocol was approved by the Danish Animal Experiments Inspectorate under the Danish Ministry of Food, Agriculture and Fisheries, and animal care followed guidelines of the National Institutes of Health.

Human material. The use of human material was approved by the Danish Ethical Committee, and kidney specimens were received from the Department of Urology at Odense University Hospital (Odense, Denmark). Renal blood vessels were isolated from patients who underwent nephrectomy for renal cancer and had given their informed written consent. Kidneys were extirpated, and intrarenal arteries (interlobar and arcuate arteries) were isolated from normal tissue (18). The study included 13 patients, with the primary diagnoses being either malign or benign tumours. The age range was from 43–82 yr old with a mean of 64 ± 3.4 yr and blood pressure of 138 ± 6.1/86 ± 4.2 mmHg.

Vascular responses and perfused mouse afferent and efferent arterioles. Mice were killed by cervical dislocation. Cortical afferent or efferent arterioles identified by appearance and location were microdissected and perfused as previously described (2). In short, the arteriole was transferred to a temperature-controlled (37°C) chamber (Warner) containing DMEM-F-12 (saturated 5% CO_2 in air) with 0.1% BSA. The segment was perfused by aspiration of the arteriole into a holding pipette (tip diameter: 14 μm) followed by cannulation with a perfusion pipette (tip diameters: 5 μm for efferent arterioles and 7 μm for afferent arterioles) and an increase in the driving pressure until the vessel opened (20–30 mmHg). Experiments were performed using an inverted microscope system (Olympus), and the luminal diameter was measured at most reactive part of the arteriole determined at the first response to high K^+. All measurements were made at the same site of the arteriole. After the perfusion was established, all experimental protocols started with a period of equilibration, and vessel viability was tested by administration of high-K^+ solution (100 mmol/l K^+) added to the bath. Concentration-response curves were obtained for increasing concentrations of K^+ in the range of 10, 15, 30, 55, and 75 mmol/l. Each concentration was applied for 3 min in the presence of phentolamine (10^{-5} mol/l) to exclude nerve-mediated α-adrenergic effects of depolarization. Finally, the contractile response to increasing concentrations of U-46619 and ANG II was tested in afferent arterioles.

High-K^+ solution contained (in mM) 45 NaCl, 70 KCl, 25 $NaHCO_3$, 1.2 $MgSO_4$, 2.5 K_2HPO_4, 1.3 $CaCl_2$, 5.5 glucose, and 10 HEPES. Solutions were equilibrated with 5% CO_2 in air, resulting in pH 7.4 with 0.1% and 1% BSA superfusate and perfusate, respectively.

Vascular responses and isometric force measurements in intrarenal human arteries. Human intrarenal arteries were isolated under a stereomicroscope and stored at 4°C until the next day in the following solution (in mmol/l): 103 NaCl, 5.4 KCl, 4.0 $NaHCO_3$, 1.5 NaH_2PO_4, 0.8 $MgSO_4$, 5.1 glucose, 0.9 Na-pyruvate, 30 Na-isethionic acid, and 5.6 HEPES 5.6 with added 10 ml/l MEM vitamin solution (M6895, Sigma), 20 ml/l MEM essential amino acid solution (M5550, Sigma), and 10 ml/l MEM nonessential amino acid solution (M7145, Sigma) (18). Human intrarenal arteries were mounted in a Halpern-Mulvany wire myograph (model 610, Danish Myo Technology, Aarhus, Denmark), and isometric force development was measured (PowerLab, AD Instruments, Colorado Springs, CO). Artery rings were incubated at 37°C in physiological salt solution, normalized, and allowed to equilibrate for 30 min. The viability of vascular smooth muscle and endothelial cells was tested by demonstrating contraction to phenylephrine (10^{-6} mol/l) and relaxation to ACh (10^{-6} mol/l), respectively. In human intrarenal arteries, the contraction induced by increasing concentrations of K^+ (20, 40, 60, 80, and 100 mmol/l) was tested in the absence and presence of the T-type blockers mibefradil (10^{-7} mol/l) and NNC 55-0396 (3×10^{-6} mol/l, Sigma-Aldrich). Each concentration of K^+ was applied for 5 min in the presence of phentolamin (10^{-5} mol/l).

Arterial blood pressure, heart rate, GFR, and renal plasma flow. Mice were anesthetized (100 mg/kg ketamine and 10 mg/kg xylazine). For measurements of arterial blood pressure and infusions of drugs, catheters consisting of a micro-renathane tip connected to polyethylene tubing were placed in the femoral artery and vein, respectively, and exteriorized through a subcutaneous tunnel from the groin to the back of the neck (3, 16). In short, the catheters was filled with heparin solution (100 IU/ml in isotonic glucose) and attached to a swivel, enabling the mice to move freely. For analgesia, mice were given subcutaneous injections of Temgesic [buprenorphinum (0.3 mg/ml, 3.75 mg/kg)]. Mice recovered for 5 days before continuous measurements of mean arterial pressure (MAP) and heart rate (HR) for 4 days. The arterial line was connected to a pressure transducer (Föhr Medical Instruments, Hessen, Germany). Data were collected using LabView software (National Instruments, Austin, TX).

GFR was measured based on a constant infusion of inulin (25). Sinistrin [inutest (25%, 10 μl/h)] was infused by connecting the venous catheter to an infusion pump (Electronic shop, University of Southern Denmark). The infusion lasted for 24 or 96 h to achieve steady state, and a blood sample (100 μl) was withdrawn from the arterial catheter at both time points. The plasma inulin concentration was determined by spectrophotometry with a modified version of the method of Gabel et al. (13). The inulin concentration was quantified by reaction by inulinase (Sigma-Aldrich). Inulin was hydrolyzed to fructose, which was converted to sorbitol by sorbitol dehydrogenase. The amount of NADH consumed in the process is proportional to the amount of inulin in the sample. NADH concentrations were measured by spectrophotometry at 340 nm (Versa Max microplate reader, Molecular Devices). GFR was calculated as the inulin infusion rate divided by the plasma inulin concentration.

In other experiments, PAH clearance was used as an estimate of renal plasma flow. PAH (20% in saline, 10 μl/h, Merck) was infused continuously for 24 and 96 h, and two blood samples (100 μl) was taken at both time points. The experiment was repeated in WT mice using 10% PAH in saline to verify that the tubular secretion maximum was not reached. Blood samples were centrifuged, and plasma was collected and stored at −80°C. PAH in plasma was measured by a colorimetric reaction with dimethylaminocinnamaldehyde solution in an acidic environment. The intensity of the color generated was measured at 545 nm after 15–30 min of incubation. Renal plasma flow was calculated as the PAH infusion rate divided by the plasma PAH concentration at steady state, during which the infusion rate equals the excretion rate.

Statistical analysis. Data are presented as means ± SE. Significance of changes was calculated by two-way ANOVA with a Bonferroni reduction for multiple comparisons and Student's *t*-test for comparison of two groups. *P* values of <0.05 were considered significant.

RESULTS

$Ca_v3.2$ mice. The contribution of $Ca_v3.2$ channels to baseline renal hemodynamics was assessed by measurements in $Ca_v3.2^{-/-}$ mice. GFR was significantly increased in $Ca_v3.2^{-/-}$ mice compared with $Ca_v3.2^{+/+}$ mice after 24 h of infusion of inulin (Fig. 1*A*) by 37% with no significant difference between sexes. There was no difference between inulin clearance measured after 24 and 96 h of infusion (data not shown). PAH clearance/renal plasma flow was not significantly changed in $Ca_v3.2^{-/-}$ mice compared with WT mice (Fig. 1*B*). PAH clearances after 24 h of infusion were 1.4 ± 0.2 and 1.7 ± 0.3 ml·min^{-1}·25 g mouse^{-1} in $Ca_v3.2^{+/+}$ and $Ca_v3.2^{-/-}$ mice, respectively, with no significant difference between sexes. In control experiments, there was no significant difference between renal plasma flows measured after 24 or 96 h of infusion or by infusion of 10% or 20% PAH at identical rates, suggesting that a steady state was present and that the tubular transport maximum was not reached by use of the higher concentration (not shown).

MAPs and HRs measured over 4 days were not significantly different between $Ca_v3.2^{+/+}$ and $Ca_v3.2^{-/-}$ mice (Fig. 1, *C* and *D*), and there were also no significant differences in HR or MAP between sexes (not shown).

The contractility of isolated arterioles was measured in response to graded depolarization by K^+ in $Ca_v3.2^{+/+}$ and $Ca_v3.2^{-/-}$ mice. In afferent arterioles, the diameter averaged 8.1 ± 0.3 and 8.1 ± 0.4 μm in $Ca_v3.2^{+/+}$ and $Ca_v3.2^{-/-}$ mice, respectively ($n = 6$ in each series). The contractile response to increasing concentrations of K^+ in afferent arterioles revealed no significant difference in contractility between WT and $Ca_v3.2^{-/-}$ animals, although a large a variance was observed in $Ca_v3.2^{-/-}$ responses at low concentrations of K^+ (Fig. 2*A*). Three of six vessels constricted to 10 mmol/l K^+; however, this response was not significant. K^+ (30 mmol/l) induced a significant constriction after 3 min of administration to afferent arterioles from both $Ca_v3.2^{-/-}$ and WT mice (Fig. 2*B*). Basal diameters of efferent arterioles were not significantly different (7.3 ± 0.3 μm in $Ca_v3.2^{+/+}$ mice and 7.0 ± 0.3 μm in $Ca_v3.2^{-/-}$ mice, $n = 8$). Depolarization-induced constrictions were significantly larger in the absence of $Ca_v3.2$: the EC_{50} value for $Ca_v3.2^{-/-}$ mice was 16 mmol/l compared with 31 mmol/l for $Ca_v3.2^{+/+}$ mice ($P < 0.05$; Fig. 2*C*). K^+ (30 mmol/l) induced a significant constriction after 3 min of administration to efferent arterioles from $Ca_v3.2^{-/-}$ mice, whereas no significant constriction was observed in WT mice (Fig. 2*D*).

$Ca_v3.1$ mice. The involvement of $Ca_v3.1$ in the regulation of baseline renal perfusion and GFR was tested in $Ca_v3.1^{-/-}$ and WT mice. Values of GFR were not significantly different (Fig. 3*A*). However, renal plasma flow was significantly elevated compared with WT mice (2.0 ± 0.1 vs. 1.5 ± 0.1 ml·min^{-1}·25 g mouse^{-1}; Fig. 3*B*).

Resting basal MAPs averaged 105 ± 4 and 104 ± 3 mmHg in WT and $Ca_v3.1^{-/-}$ mice, respectively ($n = 6$), with a pronounced circadian rhythm in both genotypes (Fig. 4, *A* and *C*); there was no significant difference between sexes. HRs in WT and $Ca_v3.1^{-/-}$ mice averaged 623 ± 19 and 592 ± 24 beats/min, respectively (Fig. 4, *B* and *D*). In male mice, HR was attenuated significantly in $Ca_v3.1^{-/-}$ mice compared with WT mice. Female mice had significantly higher HRs than male mice in both genotypes (Fig. 4*D*).

The increase in renal plasma flow could be due to a decrease in vascular resistance in the afferent and/or efferent arteriole. The possible contribution of $Ca_v3.1$ channels to arteriolar

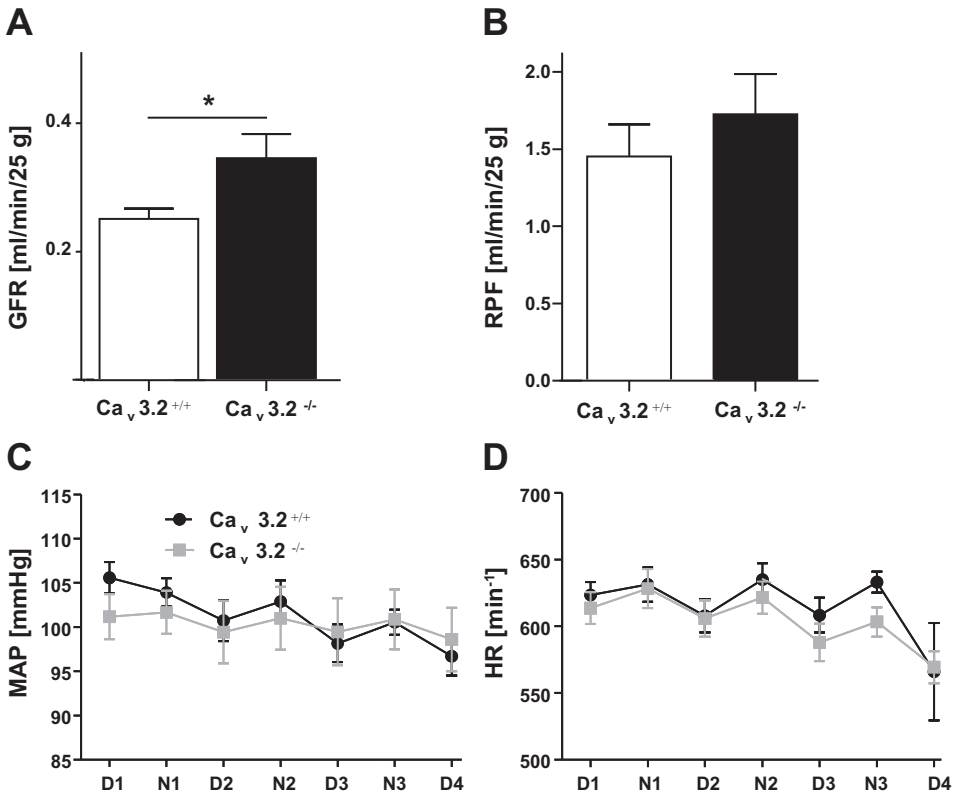

Fig. 1. Renal hemodynamics, mean arterial pressure (MAP), and heart rate (HR) in $Ca_v3.2^{+/+}$ and $Ca_v3.2^{-/-}$ mice. *A*: glomerular filtration rate (GFR) was measured based on inulin clearance using mice with chronically indwelling catheters. *$P = 0.02$. *B*: para-aminohippuric acid (PAH) clearance was used as an estimate of renal plasma flow (RPF). $P = 0.44$. *C*: MAP measured with indwelling catheters for 4 days (D1–D4) and 3 nights (N1–N3). *D*: HR measured over 4 days (D1–D4) and 3 nights (N1–N3). Data are means ± SE; $n = 12$ for inulin clearance and 7 for PAH clearance.

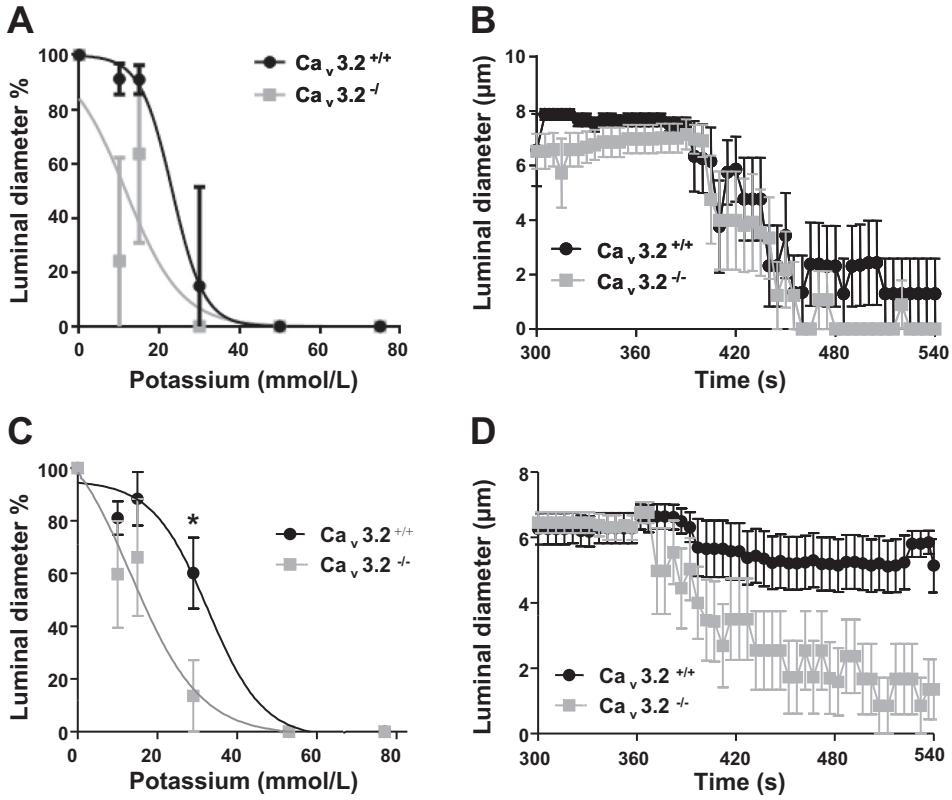

Fig. 2. Depolarization induced constrictions in afferent and efferent arterioles from $Ca_v3.2^{+/+}$ and $Ca_v3.2^{-/-}$ mice. *A*: effect on luminal diameter (in μm) in afferent arterioles in mice stimulated with 10, 15, 30, 55, and 75 mmol/l K+ every 3 min. $P = 0.37$ between genotypes. *B*: time course in afferent arterioles in mice stimulated with 30 mmol/l K^+ ($n = 4$). *C*: depolarization induced constriction by K^+ in efferent arterioles. $^*P = 0.03$ between genotypes. *D*: time course of 30 mmol/l K^+-induced constriction in efferent arterioles. Data are means ± SE; $n = 8$ efferent arterioles and 6 afferent arterioles.

constriction was studied in isolated perfused vessels. Resting basal diameters of efferent arterioles averaged 7.0 ± 0.3 and 7.0 ± 0.3 μm in WT and $Ca_v3.1^{-/-}$ mice, respectively ($n = 8$). In afferent arterioles, diameters averaged 8.6 ± 0.3 and 8.8 ± 0.5 μm in WT and $Ca_v3.1^{-/-}$ mice, respectively ($n = 6$). In all experiments, the startup protocol with high-K^+ solution elicited a significant constriction that did not differ among the genotypes. The experiment testing the response to increasing concentrations of K^+ on afferent arterioles revealed no difference in contractility between WT and $Ca_v3.1^{-/-}$ animals, with EC_{50} values of 30 mmol/l in WT animals and 24 mmol/l in $Ca_v3.1^{-/-}$ animals (Fig. 5*A*). K^+ at 30 mmol/l mediated the same degree of constriction in both $Ca_v3.1^{-/-}$ and WT animals (Fig. 5*B*). In efferent arterioles, eight individual experiments testing the effect of K^+ showed a concentration-dependent constriction with EC_{50} values of 17 mmol/l in WT animals and 19 mmol/l in $Ca_v3.1^{-/-}$ animals (Fig. 5*C*), showing no significant difference between WT and $Ca_v3.1^{-/-}$ animals. The time courses of the constriction in response to 30 mmol/l K^+ were indistinguishable (Fig. 5*D*).

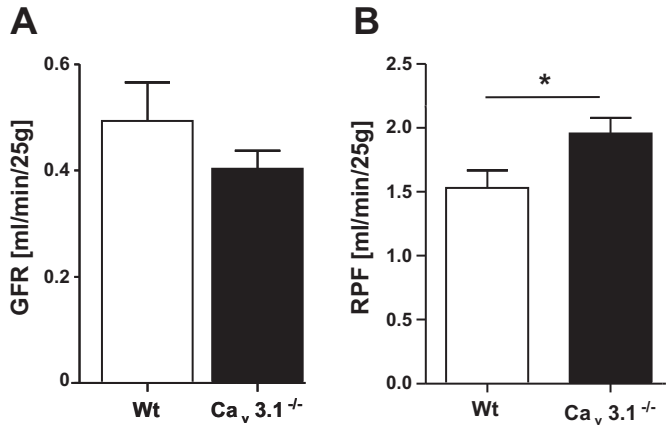

Fig. 3. Renal hemodynamics in wild-type (WT; $Ca_v3.1^{+/+}$) and $Ca_v3.1^{-/-}$ mice. *A*: GFR was measured based on inulin clearance using mice with chronically indwelling catheters. $P = 0.30$. *B*: PAH clearance was used as an estimate of RPF. $^*P = 0.02$. Data are means ± SE; $n = 14$ for inulin clearance and 7 for PAH clearance.

Human arteries. The functional involvement of T-type Ca^{2+} channels was tested in isolated human intrarenal arteries in a myograph setting after depolarization. The K^+ concentration dependently contracted human intrarenal artery rings with an EC_{50} value of 36 mmol/l. Administration of mibefradil in concentrations specific for T-type Ca^{2+} channels significantly inhibited the contraction induced by 20 mmol/l K^+ (Fig. 6*A*). Another T-type antagonist, NNC 55-0396, also inhibited the contraction significantly; however, this effect occurred at all K^+ concentrations (Fig. 6*B*).

DISCUSSION

The present results demonstrate markedly different renal hemodynamic effects of absence of $Ca_v3.2$ or $Ca_v3.1$ at similar average arterial blood pressure. The data support the conclusion that $Ca_v3.2$ preferentially lowers resistance in the efferent arteriole, thereby affecting GFR, as $Ca_v3.2^{-/-}$ animals had increased contractile responses of the efferent arteriole in response to depolarization and increased GFR with no significant change in plasma flow. In contrast, deletion of $Ca_v3.1$ channels increased renal plasma flow, whereas GFR was not changed measurably. That could be in agreement with an equal

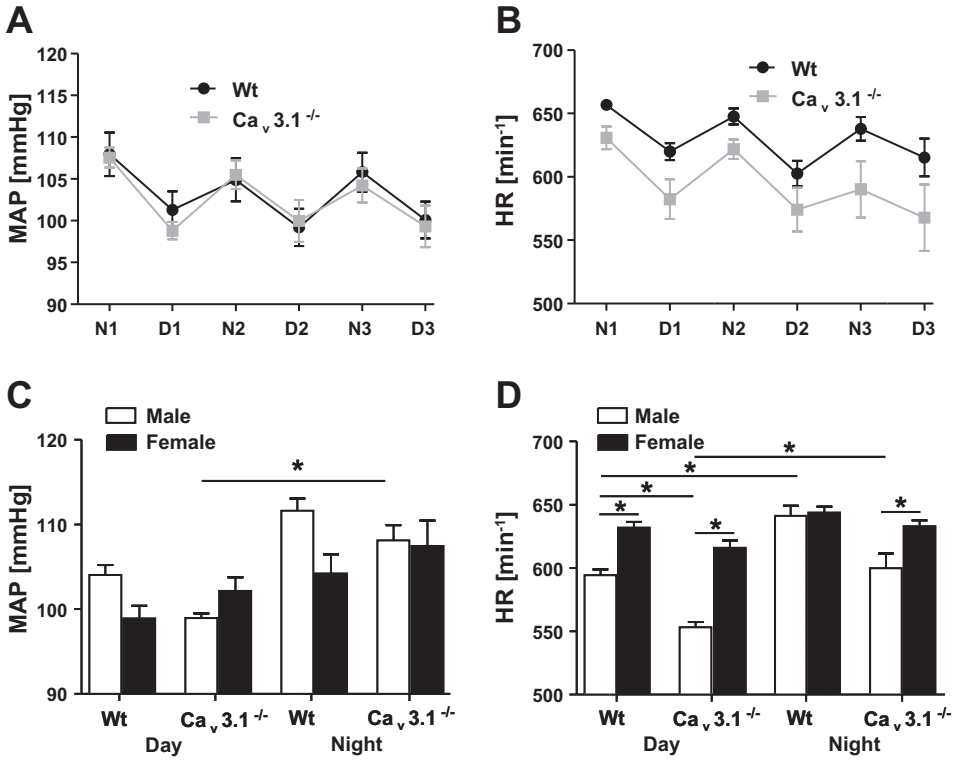

Fig. 4. MAP and HR in WT and $Ca_v3.1^{-/-}$ mice. *A*: MAP was measured with indwelling catheters over 3 days (D1–D3) and 3 nights (N1–N3). *B*: HR was measured for 3 days (D1–D3) and 3 nights (N1–N3). *C*: sex differences in average values of MAP in day and night time. *D*: sex differences in average values of HR in day and night time. Data are means ± SE; $n = 6$. $^*P \leq 0.05$.

effect on the vasculature along afferent and efferent glomerular arterioles, although that was not observed in isolated renal arterioles in vitro. Notably, these changes occurred in the absence of changes in systemic arterial blood pressure.

The present study shows that T-type Ca^{2+} channels mediate dilatation of renal postglomerular blood vessels as the constrictor response to depolarization was enhanced in efferent arterioles from $Ca_v3.2^{-/-}$ mice. There was no significant difference

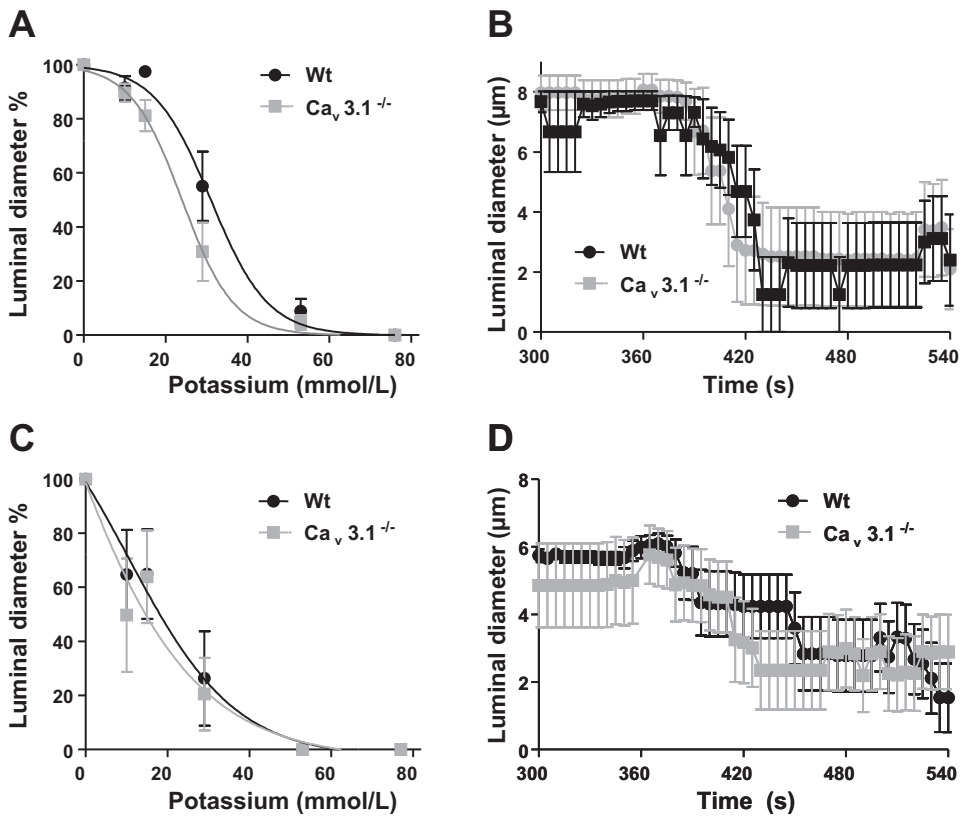

Fig. 5. Depolarization-induced constriction in mouse ($Ca_v3.1$ and WT) efferent and afferent arterioles. *A*: effect on luminal diameter (in μm) in afferent arterioles in mice stimulated with 10, 15, 30, 55, and 75 mmol/l K^+ every 3 min. *B*: time course in afferent arterioles in mice stimulated with 30 mmol/l K^+ ($n = 6$). *C*: depolarization induced constriction by K^+ in mouse efferent arterioles. *D*: time course of 30 mmol/l K^+-induced constriction in efferent arterioles in mice ($n = 8$). Data are means ± SE.

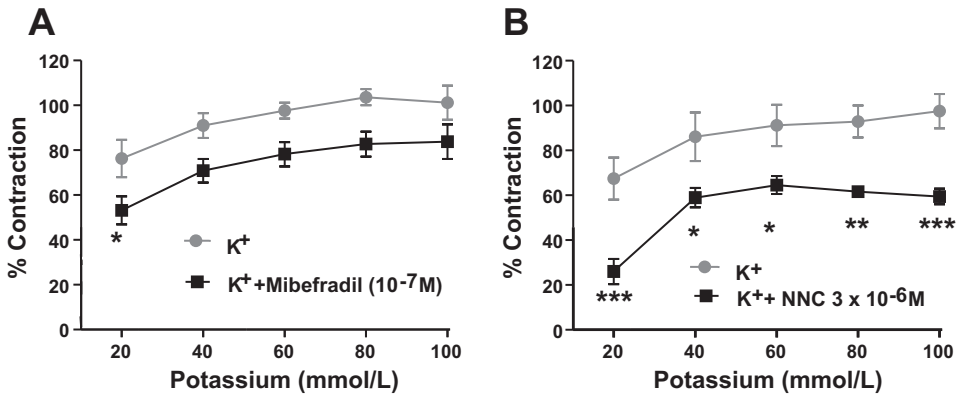

Fig. 6. Depolarization-induced constriction in human intrarenal arteries. *A*: effect of T-type Ca_v channel inhibition by mibefradil on K^+-induced dose-response contractions in human intrarenal blood vessels ($n = 7$). *B*: effect of NNC 55-0396 (NNC; T-type blocker) on K^+-induced dose-response contractions in human intrarenal arteries ($n = 6$). All data are means ± SE. $*P \leq 0.001$; $**P \leq 0.01$; $***P \leq 0.05$.

in contractility between afferent arterioles from WT and knockout (KO) animals, although a transient constriction was observed in some $Ca_v3.2^{-/-}$ mice at the lowest K^+ concentration. This supports the suggestion that the T-type Ca^{2+} channel subtype $Ca_v3.2$ mediates the secondary dilatation after depolarization-induced constriction of the mouse perfused cortical efferent arteriole (36). In agreement, inhibition of $Ca_v3.2$ by Ni^{2+} at low concentrations potentiates the constriction by changing it from a transient response to a sustained response. Coronary blood vessels from $Ca_v3.2^{-/-}$ mice show reduced relaxation after administration of ACh and nitroprusside (8). Furthermore, the dilatation of perfused efferent arterioles is blocked by inhibition not only of $Ca_v3.2$ but also of eNOS, suggesting that T-type $Ca_v3.2$ channels and NO release are involved in the vasodilatation (36). The increased contractility of efferent arterioles from $Ca_v3.2^{-/-}$ mice could account for the observed increase in GFR by elevating filtration pressure. This would suggest that under normal physiological conditions, $Ca_v3.2$ channels in postglomerular arterioles mediate a dilatation that contributes to lower glomerular pressure. The renal plasma flow was not significantly changed, and MAP was not affected by lack of $Ca_v3.2$ channels. This is in agreement with previous recordings of systolic blood pressure using tail cuffs (10). Changes in blood pressure could therefore not account for the increased GFR in KO animals. Furthermore, HR was not different between genotypes, suggesting that it is mainly $Ca_v3.1$ channels that are involved in regulating HR, as observed in the present study and in a previous study by Mangoni et al. (30).

For $Ca_v3.1$ channels, the present in vivo data are in agreement with previous in vivo pharmacological data compatible with important effects of T-type Ca^{2+} channels on renal hemodynamics. In anesthetized dogs, the T-type blocker mibefradil increased renal blood flow with no significant effect on GFR, suggesting balanced effects on afferent and efferent arterioles, whereas L-type blockage (with nifedipine) increased both renal plasma flow and GFR, suggesting a preferential afferent effect. Similarly, in spontaneously hypertensive rats, T-type blockade increased renal plasma flow more than GFR, resulting in a decrease in the filtration fraction (43). The increase in renal blood flow without an increase in GFR in response to T-type blockade has been suggested to be due to dilatation of the efferent arteriole. An in vivo study (23) using a camera probe inserted directly into the kidney revealed a relative dilation of the efferent arteriole larger than that of the afferent arteriole after administration of the T-type antagonist mibefradil, and, in agreement, T-type antagonists have been demonstrated to dilate efferent arterioles in vitro (4, 20, 21, 35, 36). However, in the present study, K^+-induced contractility responses of afferent and efferent arterioles of $Ca_v3.1^{-/-}$ mice were not significantly different compared with WT mice. This is in disagreement with previous pharmacological studies (15, 22) showing an effect of a T-type blocker on arteriolar contractility.

The different responses in vivo with increased renal blood flow in $Ca_v3.1^{-/-}$ mice with no apparent contribution of $Ca_v3.1$ in vitro in afferent arterioles could also be due to an attenuated sympathetic efferent nerve traffic that involves T-type Ca^{2+} channels. Chen and coworkers (7) investigated central sympathetic activity and T-type Ca^{2+} channels in a preparation of brain stem-spinal cord-splanchnic sympathetic nerves. They demonstrated that $Ca_v3.2$ channels were required for maintaining central sympathetic outflow, and $Ca_v3.1$ had an inhibitory effect (7). In a clinical study (19), the T-type antagonist efonidipine reduced HR and sympathetic nervous activity. Renal nerve activity was not measured directly in $Ca_v3.1^{-/-}$ animals, but since $Ca_v3.1^{-/-}$ male mice displayed a lower HR, this would be compatible with attenuated sympathetic drive, although impaired automaticity in the sinus node also might be involved.

Furthermore, the in vivo data could be affected by a changed hormonal status as T-type blockers are involved in aldosterone release (9, 37) and the T-type antagonist efonidipine lowers aldosterone plasma levels in humans (34). The aldosterone level in KO mice could therefore be expected to be lowered in KO animals. However, a potential lower aldosterone level is less likely as the reason for the observed increased GFR.

Both T-type Ca^{2+} channels ($Ca_v3.1$ and $Ca_v3.2$) have been shown to be expressed in renal glomerular vessels (17). In contrast to the expression data, a recent study (40) found no T-type Ca^{2+} currents in either afferent or efferent arteriole myocytes, but T-type Ca^{2+} currents were detected in tail arteries. T-type Ca^{2+} currents have previously been measured in preglomerular vascular smooth muscle cells (14). However, this discrepancy might be explained by the heterogeneous population of cells described by Gordienko et al. (14).

Ca^{2+} blockers remain a popular first choice drug in the treatment of essential hypertension; however, the selectivity for different Ca^{2+} channels differs, with some drugs affecting both L- and T-type Ca^{2+} channels and others affecting only

L-type Ca^{2+} channels. Combined T- and L-type blockers have been suggested to have a therapeutic advantage over selective L-type blockers by providing renoprotection due to differential expression of Ca^{2+} channels, with only T-type Ca^{2+} channels ($Ca_v3.1$ and $Ca_v3.2$) expressed in efferent arterioles (15, 22, 42). Clinical data support a renoprotective effect of combined T- and L-type blockers as it was concluded that treatment with a combined L- and T-type antagonist yields greater efficacy that a L-type antagonist in reducing blood pressure and proteinuria (32, 33, 38). The Amlodipine-to-Benidipine Changeover study showed that benidipine (a combined L- and T-type antagonist) caused a larger reduction in blood pressure and proteinuria compared with L-type treatment by amlodipine (32), suggesting a vasoconstrictor effect of T-type Ca^{2+} channels on efferent arterioles. The present data show that it is imperative to discriminate between the effects of $Ca_v3.1$ and $Ca_v3.2$ since selective abrogated function of $Ca_v3.2$ leads to increased GFR. Therefore, antagonists with a $Ca_v3.2$ preference are less likely to mediate a renoprotective effect. The renoprotective effect of combined blockers in clinical studies could be due to a preferential action on $Ca_v3.1$, since an increased renal plasma flow and a tendency to decreased GFR was found in the absence of $Ca_v3.1$ channels in mice. T-type Ca^{2+} channels are present in human resistance blood vessels (18).

The present study using pharmacological inhibitors shows that T-type Ca^{2+} channels play a significant role in contractility of human intrarenal arteries. Increased tension at low K^+ concentrations was significantly inhibited after both mibefradil and NNC 55-0396. This implies a graded contribution of Ca_v channels with T-type effects at small deviations from the resting membrane potential. This is in agreement a recent study in T-type KO mice (5) and in a pharmacological study (1) showing the involvement of T-type Ca^{2+} channels in response to small increases in perfusion pressure, which concluded that T-type Ca^{2+} channels do play a role in the myogenic response (5). In contrast to the present results with mibefradil, NNC 55-0396 inhibited the contraction induced at all K^+ concentrations, which could be due to variations between patients or, more likely, that the drug also affects L-type Ca^{2+} channels, as previously suggested (26). Furthermore, an altered function of kidney vascular segments occurs in several pathological conditions, such as diabetes and hypertension (11, 39). The present confirmation of a functional role for T-type Ca^{2+} channels in the regulation of renal function suggests that T-type Ca^{2+} channels could play a role under physiological conditions as well as in pathophysiological situations.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: A.D.T., H.A., M.C., A.T., S.W., and N.M. performed experiments; A.D.T., H.A., M.C., P.B., and P.B.H. analyzed data; A.D.T., H.A., M.C., B.L.J., P.B., and P.B.H. interpreted results of experiments; A.D.T., H.A., M.C., and P.B.H. prepared figures; A.D.T., H.A., M.C., A.T., S.W., N.M., B.L.J., P.B., and P.B.H. approved final version of manuscript; A.T., S.W., N.M., and P.B.H. conception and design of research; B.L.J., P.B., and P.B.H. edited and revised manuscript; P.B.H. drafted manuscript.

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